Molecular characterization of blaESBL-producing Escherichia coli cultured from pig farms in Ireland

Wang, J ; Gibbons, James F ; McGrath, K ; Bai, L ; Li, Fengqin ; Leonard, Finola C ; Stephan, Roger ; Fanning, Séamus

Abstract: Objectives: To characterize ESBL-encoding Escherichia coli cultured from pigs and their plasmids carrying these genes following conjugation into recipient strains. METHODS: Six ESBL-producing E. coli were recovered from faecal samples taken from pigs along with a further isolate from the environment of a farrowing house on three pig farms in Ireland. These isolates were characterized by phylogenetic grouping, MLST and ESBL genotype analyses. Conjugation experiments were carried out in broth mating assays. S1-nuclease PFGE was used to determine the plasmid profiles. Whole-genome sequences of the seven E. coli were determined and subsequently analysed. RESULTS: Phylogenetic groups and the corresponding MLST STs identified among the seven tested E. coli isolates included A/ST10, A/ST34, C/ST23 and C/ST1629. All seven isolates carried one or more high-molecular-weight plasmids and demonstrated the ability to transfer their cefotaxime resistance phenotype at high frequencies. Five transmissible plasmid replicon types were detected, including IncK/B (n = 3), IncI1 (n = 2), IncFIA (n = 1), IncFIB (n = 1) and IncN (n = 1). ESBL-encoding genes, including blaCTX-M-14, blaCTX-M-15 and blaTEM-20, were identified. CONCLUSIONS: As the first report from pig sources in Ireland, characterization of these ESBL-encoding isolates and their transmissible plasmids extends our understanding on these resistance markers from porcine E. coli.

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Molecular characterization of *bla*$_{ESBL}$-producing *Escherichia coli* cultured from pig farms in Ireland

Juan Wang[1,2], James F. Gibbons[3], Kathleen McGrath[2], Li Bai[4], Fengqin Li[4], Finola C. Leonard[3], Roger Stephan[5] and Séamus Fanning[2,4,6]

[1]College of Veterinary Medicine, Northwest A&F University, Yangling 712100, Shaanxi, China;
[2]UCD-Centre for Food Safety, School of Public Health, Physiotherapy & Sports Science, University College Dublin, Belfield, Dublin 4, Ireland;
[3]School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland;
[4]Key Laboratory of Food Safety Risk Assessment, Ministry of Health, China National Centre for Food Safety Risk Assessment, 7 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China;
[5]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 272, CH-8057 Zurich, Switzerland;
[6]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Stranmillis Road, Belfast BT9 5AG, Northern Ireland

Corresponding author- Tel.: +353-1-716 2869; Fax: +353-1-716 1147; sfanning@ucd.ie

Sir,

ESBL-producing *Escherichia coli* of porcine origin have been detected with increasing frequency during the past few years, and plasmid-mediated transfer of ESBL genes from pigs is considered to be a significant factor driving the dissemination of these markers.[1, 2] However, epidemiological data describing ESBL-producing *E. coli* in Irish food-producing animals is limited. In an earlier study, the antimicrobial resistance associated with a total of 1,126 *E. coli* cultured from 39 Irish pig farms, located in geographically distinct regions, with recorded variability in duration of in-feed antimicrobial compound use, was studied.[3] This current report describes a follow-up study, in which seven isolates that were resistant to cefotaxime were further characterized.

The corresponding serotypes; phylotypes and sequence types were determined for all seven isolates studied, using previously published protocols (**Table 1**).[4 5] The MIC for cefotaxime was determined by Etest and ESBL-encoding genes were further characterized by PCR and amplicon sequencing.[6] Conjugation experiments were carried out on each isolate individually to assess their ability to transfer cefotaxime resistance.[7] Antimicrobial susceptibility testing was performed on the *E. coli* and their associated transconjugants by agar disc diffusion according to the criteria of CLSI.[8] Plasmid analysis included replicon typing and S1-nuclease PFGE molecular profiling methods.[7] WGS was undertaken for all seven isolates using the Illumina HiSeq 2500 platform. The core-genome based phylogeny was determined by Harvest Suite and a maximum composite likelihood phylogenetic tree was generated with MEGA version 6 software using *E. coli* MG1655 as reference.[9, 10] All genomic data was submitted to EMBL and Accession numbers are listed in **Table 1**.

The source origins of all seven ESBL-positive *E. coli* are shown in **Table 1** and these were composed of four different serotypes that could be assigned into two phylogenetic groups and four sequence types. Four of the seven isolates were identified as ST10 and ST34, belonging to the CC10, which is one of the largest clonal complexes identified within the *E. coli* MLST database. These isolates were cultured from two farms that were geographically located in separate regions, Cork and Roscommon. The latter ESBL-positive *E. coli* possessed $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-20}}$ –encoding genes (**Table 1**). The important contribution of the ST10 type to the spread of resistance is a very recent observation; wherein previously this sequence type was reported both in humans and/or animals in Spain, Italy, Germany and other European countries.[11-13] Similarly, members of the ST23 clonal complex have also been observed to be an important source of $bla_{\text{CTX-M}}$ genes.[13] In our study, two ST23 isolates were identified from a single farm and carried $bla_{\text{CTX-M-14}}$. The same ESBL-genotype was also linked with a ST1629 from a separate group of animals on the same farm in this study. To the best of our knowledge, this is

the first reported identification of ST1629 carrying ESBL genes from food-producing animals.

All isolates transferred their cefotaxime resistance marker to a susceptible *E. coli* 26R 793 (Rif^r) recipient with transfer rates ranging from 3.2×10^{-4} to 8.5×10^{-1}. The MIC determined for cefotaxime by Etest recorded values ranging from 8- to >32- mg/L. The bla_{ESBL} genes transferred *via* conjugation were subsequently confirmed as bla_{TEM-1}, bla_{TEM-20}, $bla_{CTX-M-14}$, and $bla_{CTX-M-15}$ (**Table 1**). The first study to report on the prevalence of ESBL-positive Enterobacteriaceae in Ireland, was conducted in 2003, but was limited to clinical settings.[14] These data showed that $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$ genes were the most common ESBL genotypes identified in those bacterial isolates cultured from Irish hospitals.[15] The same genotypes were also recovered in Ireland from *Salmonella enterica* from humans.[16] A bla_{TEM-20} genotype was identified in our study and this was previously identified in a *Salmonella enterica* (serovar Java) cultured from Irish beef in 2006.[16]

Plasmid profiles were determined by S1-nuclease treatment followed by PFGE. All isolates contained detectable large molecular-weight plasmids. Heterogeneity among the profiles was a common featured noted, although most of the plasmid profiles were related among strains isolated from same geographical locations (**Figure S1**). Following conjugation, the plasmid profiles were again determined by S1-nuclease PFGE for the transconjugants recovered. Two isolates from Cavan, F1B2 and F1L4 possessed one transmissible plasmid (of approximately 100-kbp). Two plasmids were transferred in the case of isolate F1L3, and which were approximately 120- and 200-kbp in size. From the isolates F13P5 and F13P4, only one transmissible plasmid was detected in each case, of between 70- and 80-kbp, respectively. The remaining two isolates, F25OS1 and F25P5, possessed two large transmissible plasmids. Three replicon types (including IncFIA, IncFIB and IncK/B) were detected in *E. coli* F1L3 and all three Inc-types were also identified from the corresponding transconjugant. The same Inc-type profile was also noted in *E. coli* F1L4, and IncK/B was the sole type detected following conjugation (**Table 1**). In the *E. coli* isolates denoted as F25OS1 and its transconjugant, IncI1 and IncN types were identified. Bacterial isolates, F13P5 and F13P4 that were originally cultured from piglets in Cork, were positive for an IncFIB type plasmid. The IncK/B and IncI1 types were detected in conjugative mating pairs for F1B2 and F25P5, respectively (**Table 1**).

In an earlier report, conjugative pCT-like plasmids (of the IncK group, approximately 100-kbp in size, accession no. FN868832) were determined to be important vectors contributing to the dissemination of $bla_{CTX-M-14}$ genes in the United Kingdom, Europe, Australia, and Asia.[17, 18] In our study, three isolates carried these $bla_{CTX-M-14}$ genes in association with IncK/B type

plasmids. Of these, one transmissible plasmid of approximately 100-kbp was also identified. Furthermore, according to the WGS data, 78%, 78% and 95% of the plasmid genetic backbone of pCT were also present in all three *bla*$_{CTX-M-14}$-producing isolates from a single farm, namely F1L3, F1L4 and F1B2, respectively. Based on these observations, it is tempting to speculate that pCT–like plasmids may be contributing to the spread of *bla*$_{CTX-M-14}$ genes among these pig herds in Ireland. Further sequence analysis and reconstruction of these plasmids would be required to support this conclusion, a feature that will be the subject of a separate report.

In order to assess the genetic relationships between these ESBL-producing *E. coli* isolates, a phylogenetic tree based on their core-genome was generated with MEGA6 software. **Figure-1** shows that the seven ESBL strains were clustered into three different groups. The seven *E. coli* clustered according to their ST. Four ST10 complex (including ST34) *E. coli*, which were cultured from two locations in Ireland, formed a closely related cluster with *E. coli* MG1655. All of these isolates were determined to be phylogroup A isolates. *E. coli* of sequence type ST1629 are rarely reported, and this isolate was separated by a large genetic distance when compared with the other two clusters identified. Furthermore, analysis of the genomic data also demonstrated that these *E. coli* shared several common virulence factors, such as *fimFGH* (type I fimbriae, Adherence), *ibeBC* (CFA/I fimbriae, Adherence), *ehaB* (AIDA-I type, Autotransporter), *espL/X* (Non-locus-of-enterocyte-effacement encoded type-three-secretion-system effectors) and *hlyE/clyA* (hemolysin/cytolysin A, Toxins) [**Table S1**, available as Supplementary data at *JAC* Online].[19]

In conclusion, our findings documented the characterization and potential for dissemination of their *bla*$_{CTX-M-}$encoding genes from *E. coli* cultured from pig farms in Ireland. The ST10 complex *E. coli* from two different locations, appeared to demonstrate an association with *bla*$_{CTX-M-15}$ and *bla*$_{TEM-20}$ genes. Isolates of ST23 and ST1629 sequence types from the same location were associated with a *bla*$_{CTX-M-14}$ gene. All ESBL-encoding genes studied could be transferred to a recipient bacterium *via* conjugation. WGS data showed that bacterial isolates belonging to the ST10 complex in this collection were closely related to *E. coli* MG1655, and that pCT-like plasmids identified may contribute to the transmission of *bla*$_{CTX-M-14}$ genes. Further studies are required to evaluate potential zoonotic links between ESBL-producing *E. coli* from pigs and human cases of infection.

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